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ADSORPTION OF ROTENONE AND COPPER SULFATE BY VARIOUS CONCENTRATIONS OF CLAY AND LOAM AS DETERMINED BY TOXICITY ON DAPHNIA

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by

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CHAPTER I

INTRODUCTION

The purpose of this study was to determine if either suspended particles of clay or loam adsorb rotenone or copper sulfate thus influencing their toxicity upon aquatic organisms.

In an ever increasing number federal, state and local governments as well as other organizations are using copper sulfate and rotenone to control life in ponds and other bodies of water. The use centers mostly around local recreational areas and fish management on public and small farm ponds. One of the problems confronting many of these agencies is that the method which may give good results for one locality will not produce the same good results elsewhere. There is need for more information concerning the action of both copper sulfate and rotenone so they may be used to better advantage in the future. Is it possible that some factor which has not been investigated is adsorbing the poison making it unavailable for reaction with aquatic organisms? The following investigation was based on this possibility. Clay and loam were chosen to study because they are most frequently found in the rivers and lakes as well as farm ponds, which have been coming into prominence the past few years.

The problem was, do either suspended particles in water of clay or loam adsorb copper sulfate or rotenone? The hypothesis of this problem was that if poison is adsorbed on suspended particles, the poison is not free to react with the aquatic organism and cause death. Working under this hypothesis, the more suspended materials that one encounters, the less poison would be available for reaction with the aquatic organism and thus it would take it longer to die. If this hypothesis is correct, the death time (absence of heart beat and movement of thoracic appendages) for the Daphnia (selected as the organism for use in this study) should increase as the concentration of clay or loam is increased.

This paper will present a brief account of previous work which relates to the problem under study, the method of approach involved, data obtained and its implications. This will be followed by a short discussion and summary of the material.

CHAPTER II

HISTORY OF THE PROBLEM

Much work has been done concerning the action and possible use of rotenone. It first gained prominence as a possible insecticide. In recent years its use for fish management has become important.

Various experiments show that the rough fish, buffalo (*Ictiobus sp.*) and carp (*Cyprinus canpio*), have less resistance to rotenone than game fish.[1] The action of rotenone is induced suffocation due to constriction of the capillaries in the gills.[2]

Until Howard L. Hamilton worked on the action of rotenone upon aquatic crustaceans, apparently little consideration was given to the possibility that the use of rotenone interfers with the food chain of aquatic life. In the discussion by the above named author, it was noted that macrocrustaceans were much more sensitive to rotenone than rough fish; however, the eggs of these crustaceans were not

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[1] Howard L. Hamilton, "The Biological Action of Rotenone on Fresh Water Animals, "*Proceedings of the Iowa Academy of Science* Fifty-Fifth Annual Session Held At Indianola (Indianola, Iowa: Published by State of Iowa, 1941, pp. 467-479).

[2] Hamilton, Ibid., p. 467.

susceptible and if the stock fish were not immediately dependent upon aquatic life for food, the damage to the food chain would be only temporary.[1]

Copper sulfate has been used to rid water of rough fish; it no doubt is more frequently used to rid waterways of certain types of algae, especially the blue-green. It has been observed that 3 ppm (parts per million) of copper sulfate is sufficient to destroy most rough fish.[2] Also a large number of aquatic insects and practically all phytoplankton and zooplankton are destroyed.[3] Copper sulfate has a wide range of toxic effect on various aquatic organisms, but fish once poisoned with it do not recover in fresh water.[4]

Several factors besides the poison used appear to influence the death rate of Daphnia. John Breukelman using mercuric chloride (0.0003N and 0.01N) and Daphnia magna Straus 1820 found resistance to the poison increased as the organism grew older, and the females were more resistant than

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[1] Ibid., p. 478.

[2] James Catt, "Copper Sulphate in the Elimination of Coarse Fish," Trans. American Fish Soc., Vol. 64 (1934), pp. 276-279. Biological abstracts 1937.

[3] Ibid.

[4] David L. Belding, "Toxicity Experiments with Fish in Reference to Trade Waste Pollution," Trans. American Fish Soc. Vol. 57 (1927) pp. 100-119. Biological abstracts.

the males, while the parthenogenetic form was intermediate.[1] The author suggested that the difference was due to variations in the metabolic rate.

The metabolic rate, velocity of aging, heart rate and susceptibility were all noted, by some authors, to vary directly in the same direction as the water temperature in which the Daphnia were living.[2] It is possible that the life span of Daphnia can be controlled. High temperatures result in a shorter life span, while cooler environment favors a longer average life span.[3] Other authors have found that the reproductive rate and growth of Daphnia longispina O. F. Müller 1785 decrease with dilute culture media; however, the length of life was increased.[4]

Research and the material gathered have been devoted primarily to the effects of rotenone and copper sulfate on

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[1]John Breukelman,"Effects of Age and Sex on Resistance of Daphnids to Mercuric Chloride," Science, (1930) p. 302.

[2]John W. Mac Arthur, John W., and William H. T. Baillie, "Metabolic Activity and Duration of Life. II Metabolic Rate and Their Relation to Longevity in Daphnia magna," Journal of Experimental Zoology, (1929) Vol. 52, pp. 243-258. Biological abstracts, 1930.

[3]Ibid.

[4]Lester Ingle, "Effects of Environmental Conditions on Longevity," Science, Vol. 78 (1933) pp. 511-513.

aquatic organisms and the concentrations used to destroy them. However, little consideration has been given the possibility of the poison being adsorbed on suspended materials in the water, thus making it to a degree unavailable to poison the aquatic organisms.

CHAPTER III

MATERIALS AND METHODS

Collection and preparation of loam and clay. The collection of clay and loam from ponds was carried out on the seventh of October, 1956. The two bodies of water used were located approximately five miles east of Chariton, Iowa in Lucas county. Clay samples were collected from City Lake, Range 21 west: Township 72 north: section 27. The samples were taken from the northwest shore of the lake about 2 to 3 feet from the shoreline. The mud or loam sample was collected the same day from Red Haw Lake, Range 21 west: Township 72 north: section 34. In this case the sample was taken from the north side of the lake directly below the boat docks, about four feet from the shore.

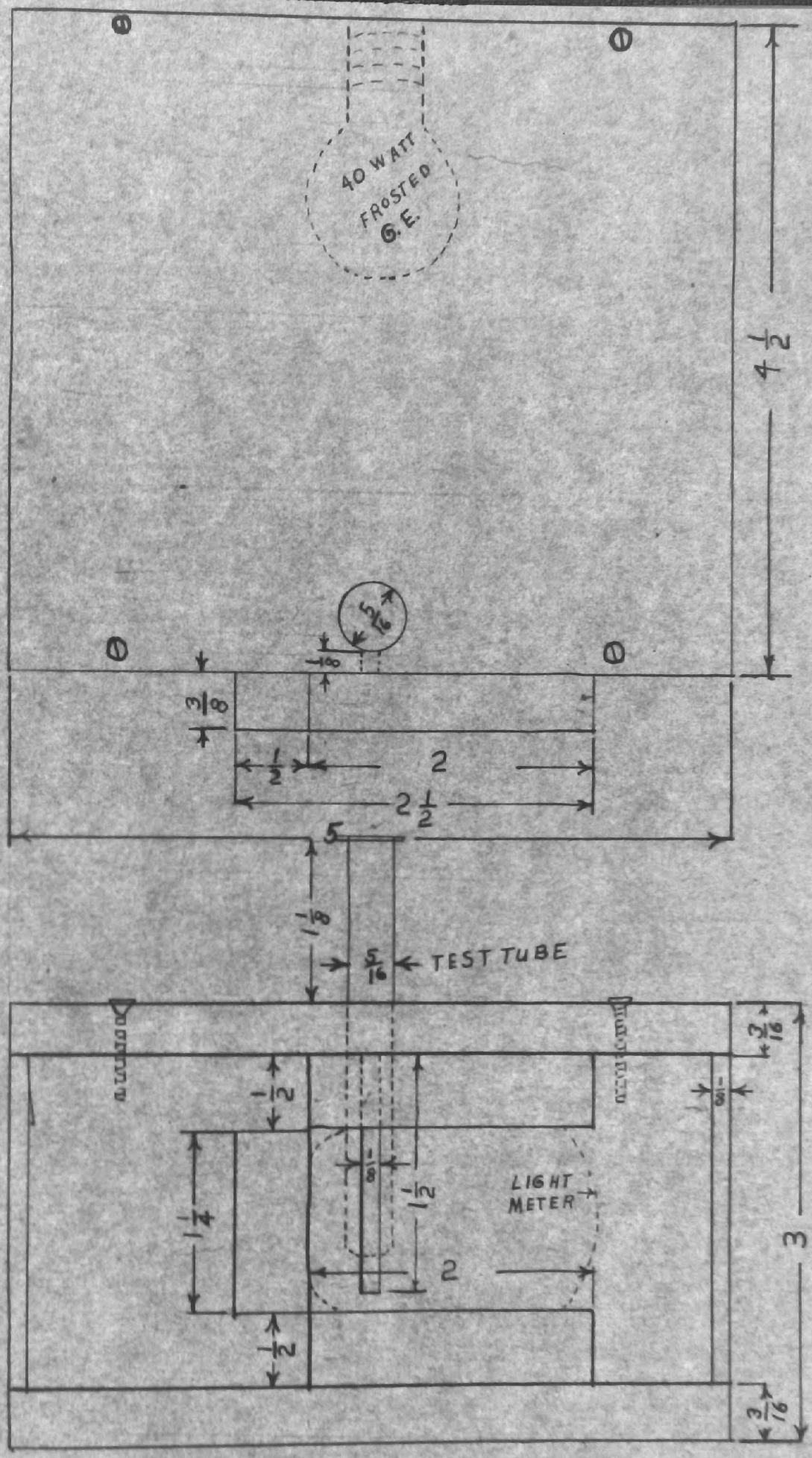
The samples of loam and clay were placed in separate jars. Later they were transferred to flat dishes for air drying. On December 1, 1956 the samples of clay and loam were removed from the dishes and sealed in bottles for storage. At the same time some of each was pulverized with mortar and pestle. After pulverizing, the samples were run through a fine mesh screen (400 squares per square centimeter). The pulverized materials were placed into separate containers for storage.

Turbidity meter. (See figure number 1) The turbidity meter used consisted of the following. Inside a small wooden box 6 inches by 9 inches by 10 inches was placed a forty watt General Electric frosted light bulb. On the 9 inch side a slit 3/8ths of an inch in width and 5 inches long was made. A hole 5/8ths of an inch in diameter was drilled in the lid of the box to allow a 6 inch test tube to be inserted between the slit and the light bulb. On the outside of the box and directly in front of the slit a Weston Master II Exposure Meter was placed (the type of light meter used for photography work).

Stirring apparatus. In order to insure a constant amount of stirring, a stirring machine was constructed. It consisted of a small electric motor (from a hair drier), and to this motor a glass rod was attached. The amount of stirring for each sample was then timed with a watch.

Method of preparing poison solution. The copper sulfate solution was prepared by weighing out 20 grams of Merck reagent cupric sulfate fine crystals. This was added to 200 milliliters of distilled water and stirred until all of the crystals were in solution.

Pro-Noxfish (this is a product of the S. B. Penick and Co. of New York and is equivalent to 5% emulsifiable rotenone) was obtained from the Iowa State Conservation



TURBIDITY METER

HALF SIZE

Commission. This solution is being used in fish work for the state. Several concentrations were tried before a concentration which killed the Daphnia between 10 and 40 minutes was obtained. The more concentrated solutions killed too rapidly and lower concentrations killed too slowly, causing too much variability in the death time of the Daphnia. The stock solution used in this experiment was made by mixing four-tenths (0.4) milliliter of Pro-Noxfish with 249.5 milliliters of distilled water, which gave a 0.0008% concentration of rotenone.

Organisms used. Daphnia pulex de Geer 1778 were obtained from the General Biological Supply House in Chicago, Illinois. The specimens were from two to three weeks old.

Procedure of the experiment. The following outline of steps was followed in each of the adsorption toxicity experiments performed:

1. The amount of clay or loam was weighed on a balance.
2. The amount of distilled water was measured to give one of the following concentrations per 50 ml.: 5 mg. 10 mg. 20 mg. 40 mg. or 80 mg.
3. Number one and two above were placed in a 50 ml. test tube: to this was added enough stock solution of rotenone to give 0.5 ml. of stock

solution for every 49.5 ml. of water used. In the case of the copper sulfate solution 5 ml. of the stock solution were used for every 20 ml. of water used.

4. The three materials above were stirred for one minute.
5. The mixture from number 4 above was then poured through a filter paper into another 50 ml. test tube.
6. Twenty-five milliliters of the filtrate from number 5 was then placed in a petri dish.
7. To the petri dish in number 6 above 10 Daphnia were added.
8. The time was noted from the introducing of Daphnia to the time that 5 (half of the sample organisms) were not swimming.
9. The time was noted also from the addition of Daphnia to the time 5 (half of the sample organisms) were dead (determined by use of a hand lens and the absence of heart beat and movement of thoracic appendages on the part of the Daphnia).

Control used. A control for each of the experimental steps performed was maintained: the above procedure was used

except that no poison (either of rotenone or copper sulfate) was used.

CHAPTER IV

STANDARDIZATION

The empirical method was used to determine the natural suspension of clay and loam in the ponds used. The findings of these preliminary investigations noted in this chapter were used as a base line in the primary problem.

Preliminary investigation with the turbidity meter. On March 16, 1957, some of the following things were noted concerning the reading of the turbidity meter. Without a test tube in the position between the forty watt bulb and the light meter, the meter reading was 170. (These readings are for comparison and need not have a measurement attached to them.) When an empty test tube was placed in the turbidity meter, the reading on the meter was 150. If this same test tube was filled with distilled water and replaced in the turbidity meter, the reading was 300.

Effect of stirring. On March 16, 1957 samples of water from Red Haw Lake and from City Lake were obtained and run through the turbidity meter without stirring. Three samples from each lake were used. In sample number 1 from Red Haw the light meter reading was 300, in sample number 2 the reading was 300, and in sample number 3 the reading was 300. From the City Lake the following readings were

obtained: in sample number 1 the reading was 280, in sample number 2 it was 310 and in sample number 3 the reading was 290. Each of these samples has a reading which is very near that of distilled water, yet when obtained they were not clear. The lakes where the samples were secured are about 5 miles from the place where the tests were carried on and during the time between the picking up of the samples and the testing of them, the suspended materials started to settle out. On occasion it was noted the suspended materials congregated near the bottom of the sample. Since there is a natural stirring up in the lakes by different organisms moving about, by the wind, and by the currents, it was decided to stir up the samples artificially before testing them. On later cases where stirring was applied before testing, the meter readings were lower.

Effect of water temperature on turbidity meter. The temperature of the water from the lakes was noted to range between 18°C. and 30°C. when measured at the laboratory.

Distilled water was heated to boiling and tested; it was found that the air bubbles had a slight influence on the turbidity meter reading as did the condensation of steam. However, at the temperature that the experiments were performed the effect of temperature on the turbidity reading was nil.

Comparative turbidity of City and Red Haw Lakes. Three samples were taken from each of the lakes every time collections were made. The samples were collected in the following manner. The cork from a six inch test tube was removed; the test tube was closed with the thumb and then placed under the surface of the water; the thumb was removed from the mouth of the test tube and the tube was allowed to fill with water; when the tube was filled with water, the cork was replaced. The collections were made in the morning between the hours of eight o'clock and ten o'clock. Each sample was tested in the turbidity meter as it was brought from the lakes; it was then stirred for 30 seconds and tested again; it was then left standing for 5 minutes and tested a third time. In each case the temperature of the water was noted. Table I gives the comparative readings for City Lake, and Table II gives the comparative readings for Red Haw Lake.

In reading the tables, the date given is to be used until a new date is given. The higher the reading the less suspended material there was in the sample.

Method of obtaining turbidity near that found in the lakes. To exactly reproduce the turbidity found in the two different lakes would be almost impossible. As it appears the turbidity of the lakes differs at different times, and

TABLE I

COMPARATIVE TURBIDITY OF WATER SAMPLES FROM CITY LAKE, LUCAS COUNTY, IOWA

Date	Temperature	Reading before stirring	Reading after stirring	Reading 5 minutes after stirring
3/23/57	20°C.	280	290	300
	20°C.	300	280	300
	20.5°C.	300	270	270
3/30/57	21°C.	300	275	290
	20°C.	280	290	280
	21°C.	290	250	250
4/13/57	21.5°C.	200	190	200
	21°C.	200	275	200
	21°C.	210	200	210
5/4/57	24°C.	300	275	280
	23°C.	305	295	300
	23°C.	310	305	305
5/11/57	19°C.	295	290	290
	19°C.	290	280	300
	19°C.	280	275	290
5/18/57	20°C.	300	295	300
	19.5°C.	310	290	305
	19.5°C.	295	290	300
Arithmetic Mean	20.66°C.	280.27	273.05	276.11

Note: Where the reading went over 300, particles, which were white in color, were noted in the water.

TABLE II

COMPARATIVE TURBIDITY OF WATER SAMPLES FROM RED HAW LAKE, LUCAS COUNTY, IOWA

Date	Temperature	Reading before stirring	Reading after stirring	Reading 5 minutes after stirring
3/23/57	19.5°C.	300	250	275
	19.5°C.	300	250	275
	19.5°C.	300	260	300
3/30/57	20°C.	290	250	275
	20°C.	200	140	160
	20°C.	280	225	260
4/13/57	18.5°C.	250	250	275
	18.5°C.	275	275	275
	19.5°C.	260	250	250
5/4/57	21.5°C.	300	290	290
	21.5°C.	320	310	310
	22°C.	300	290	290
5/11/57	19°C.	310	310	320
	19.5°C.	310	305	310
	19.5°C.	300	305	305
5/18/57	21°C.	315	305	305
	20°C.	300	305	305
	20°C.	310	300	300
Arithmetic Mean	19.97°C.	290	270.55	282.22

Note: Where the reading went over 300, particles, which were white in color, were noted in the water.

even in different samples at the same time.

In the reproduction of the turbidity found in the lakes, only distilled water and either clay or loam were used. The size of the particles of these materials varied from minute up to 0.05 centimeter in diameter, since both were run through a fine mesh screen (400 squares per square centimeter).

The following mixtures or their equivalents were made for both the loam and clay: 5 mg., 10 mg., 15 mg., 20 mg., 25 mg., and 30 mg., per 50 ml. of distilled water. Each of these was stirred for a period of one minute, then put into the turbidity meter and the reading taken. They were allowed to set for 5 minutes, then another reading was taken: after another period of 5 minutes, or a total of 10 minutes from the time of stirring, a third reading was taken. Table III shows comparative turbidity of different concentrations of clay, and Table IV shows the comparative turbidity for various concentrations of loam.

The reading of the first sample on Table III would be as follows: On the 6th of June 1957, 5 mg. of clay per 50 ml. of water immediately after stirring gave a reading of 290, 5 minutes after stirring the reading was 295, and 10 minutes after stirring the reading was 275.

TABLE III

COMPARATIVE TURBIDITY OF VARIOUS CONCENTRATIONS OF CLAY IN DISTILLED WATER

Date	Concentration per 50 ml.	0 minutes after stirring	5 minutes after stirring	10 minutes after stirring
6/6/57	5 mg.	290	295	275
	10 mg.	275	290	290
	15 mg.	275	275	280
	20 mg.	270	280	280
	25 mg.	260	280	280
	30 mg.	260	270	270
6/7/57	5 mg.	290	300	295
	10 mg.	290	280	285
	15 mg.	275	275	275
	20 mg.	260	265	265
	25 mg.	260	275	275
	30 mg.	250	260	260
6/8/57	5 mg.	290	300	290
	10 mg.	290	285	290
	15 mg.	275	250	275
	20 mg.	260	280	280
	25 mg.	255	270	275
	30 mg.	240	260	270
6/10/57	5 mg.	305	305	305
	10 mg.	285	290	280
	15 mg.	270	280	275
	20 mg.	265	270	270
	25 mg.	245	255	265
	30 mg.	235	260	260
6/11/57	5 mg.	305	295	295
	10 mg.	280	290	300
	15 mg.	280	285	280
	20 mg.	265	280	265
	25 mg.	265	275	280
	30 mg.	230	250	250

TABLE III (Continued)

Date	Concentration per 50 ml.	0 minutes after stirring	5 minutes after stirring	10 minutes after stirring
6/12/57	5 mg.	290	280	280
	10 mg.	270	280	280
	15 mg.	275	280	285
	20 mg.	265	265	250
	25 mg.	225	250	265
	30 mg.	225	250	255
Arithmetic Mean	5 mg.	295	295.83	290
	10 mg.	281.66	281.66	287.5
	15 mg.	275	274.16	278.33
	20 mg.	264.16	273.33	268.33
	25 mg.	251.66	267.5	273.33
	30 mg.	240	258.33	260.83

TABLE IV

COMPARATIVE TURBIDITY OF VARIOUS CONCENTRATIONS OF LOAM IN DISTILLED WATER

Date	Concentration per 50 ml.	0 minutes after stirring	5 minutes after stirring	10 minutes after stirring
6/6/57	5 mg.	295	295	295
	10 mg.	290	300	300
	15 mg.	300	305	305
	20 mg.	270	270	270
	25 mg.	250	265	275
	30 mg.	225	225	240
6/7/57	5 mg.	290	280	290
	10 mg.	290	280	295
	15 mg.	275	285	295
	20 mg.	275	280	280
	25 mg.	260	275	275
	30 mg.	230	250	250
6/8/57	5 mg.	295	280	290
	10 mg.	290	295	280
	15 mg.	260	270	275
	20 mg.	255	260	275
	25 mg.	250	245	250
	30 mg.	225	250	260
6/10/57	5 mg.	305	305	305
	10 mg.	290	305	305
	15 mg.	290	300	305
	20 mg.	280	295	290
	25 mg.	265	280	290
	30 mg.	250	260	275
6/11/57	5 mg.	295	290	290
	10 mg.	270	280	275
	15 mg.	250	265	275
	20 mg.	250	250	265
	25 mg.	240	250	275
	30 mg.	235	255	260

TABLE IV (Continued)

Date	Concentration per 50 ml.	0 minutes after stirring	5 minutes after stirring	10 minutes after stirring
6/12/57	5 mg.	290	295	295
	10 mg.	280	295	280
	15 mg.	265	270	265
	20 mg.	265	265	275
	25 mg.	240	250	255
	30 mg.	200	225	240
Arithmetic Mean	5 mg.	295	290	294.16
	10 mg.	285	292.5	289.16
	15 mg.	273.33	282.5	286.66
	20 mg.	265.83	270	275.83
	25 mg.	250.43	260.43	270
	30 mg.	227.5	244.16	254.16

CHAPTER V

OBSERVATIONS AND INTERPRETATION OF DATA

The observations and data obtained have been put forth in the following tables: Tables V, VI, VII, and VIII. The information from these four tables has been summarized and the probable error and standard deviation have been calculated in Tables IX and X. In reading the tables it should be noted that when temperature and date are given, that same temperature and date are to be used until a new temperature and date are given. The reading for the first line on Table V would be as follows: on August 3, 1957 with the temperature of water 27°C. and with zero mg. of clay per 50 ml. of water, it took 13 minutes until 5 Daphnia were not swimming, it took 16 minutes until 5 Daphnia were dead.

Tables IX and X show that the range of average death time did not exceed 2 minutes in any case, regardless of which combination of loam, clay, rotenone or copper sulfate was used. This tends to indicate that very little if any of the poison was adsorbed upon the suspended particles of clay or loam. If either of the poisons were being adsorbed, one would expect a gradual steady increase in death time as the concentration of suspended materials increased. In three cases, (rotenone and clay, copper sulfate, clay and loam) there was definitely no gradual increase in death time. In

TABLE V

Daphnia DEATH RATE IN VARIOUS CONCENTRATIONS OF CLAY IN DISTILLED WATER, USING ROTENONE AS POISON

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming *	Time until 5 *Daphnia* were dead*
8/3/57	27°c.	0 mg.	13	16
		0 mg.	14	20
		5 mg.	17	22
		5 mg.	12	19
		10 mg.	19	26
		10 mg.	20	32
		20 mg.	16	22
		20 mg.	18	26
		40 mg.	27	39
		40 mg.	19	38
		80 mg.	19	31
		80 mg.	21	35
8/7/57	22°c.	0 mg.	31	53
		0 mg.	29	52
		5 mg.	24	36
		5 mg.	24	45
		10 mg.	33	45
		10 mg.	32	46
		20 mg.	36	40
		20 mg.	33	47
		40 mg.	34	48
		40 mg.	38	51
		80 mg.	34	45
		80 mg.	35	42
8/7/57	24°c.	0 mg.	24	36
		0 mg.	18	26
		5 mg.	25	41
		5 mg.	17	29
		10 mg.	21	33
		10 mg.	25	39
		20 mg.	16	22
		20 mg.	23	38
		40 mg.	26	38
		40 mg.	27	44
		80 mg.	24	33
		80 mg.	19	31

* All time is to the nearest minute

TABLE V (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/8/57	24°C.	0 mg.	22	37
		0 mg.	21	38
		5 mg.	20	29
		5 mg.	23	26
		10 mg.	20	38
		10 mg.	24	43
		20 mg.	23	32
		20 mg.	25	45
		40 mg.	25	38
		40 mg.	22	36
		80 mg.	28	38
		80 mg.	24	34
8/8/57	26°C.	0 mg.	21	26
		0 mg.	25	28
		5 mg.	16	23
		5 mg.	34	43
		10 mg.	21	26
		10 mg.	15	20
		20 mg.	16	20
		20 mg.	12	15
		40 mg.	13	15
		40 mg.	36	45
		80 mg.	28	30
		80 mg.	16	21
8/12/57	28.5°C.	0 mg.	10	14
		0 mg.	12	17
		5 mg.	8	13
		5 mg.	12	18
		10 mg.	12	14
		10 mg.	8	13
		20 mg.	9	13
		20 mg.	10	15
		40 mg.	10	16
		40 mg.	11	15
		80 mg.	10	16
		80 mg.	8	14

* All time is to the nearest minute.

TABLE V (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/14/57	28°C.	0 mg.	13	24
		0 mg.	19	40
		5 mg.	18	29
		5 mg.	18	34
		10 mg.	21	36
		10 mg.	22	38
		20 mg.	17	27
		20 mg.	33	45
		40 mg.	20	26
		40 mg.	28	37
		80 mg.	19	37
		80 mg.	21	42

* All time is to the nearest minute.

TABLE VI

DAPHNIA DEATH RATE IN VARIOUS CONCENTRATIONS OF CLAY IN DISTILLED WATER, USING COPPER SULFATE AS POISON

Date	Temperature	Concentration per 50 ml.	Time until 5 Daphnia were not swimming*	Time until 5 Daphnia were dead*
7/12/57	--	0 mg.	10	18
		0 mg.	12	17
		5 mg.	14	18
		5 mg.	14	18
		10 mg.	11	15
		10 mg.	11	15
		20 mg.	16	20
		20 mg.	11	18
		40 mg.	12	16
		40 mg.	14	16
		80 mg.	15	16
		80 mg.	11	16
8/5/57	20°C.	0 mg.	16	29
		0 mg.	18	28
		5 mg.	15	18
		5 mg.	15	22
		10 mg.	15	29
		10 mg.	16	26
		20 mg.	17	29
		20 mg.	17	24
		40 mg.	18	26
		40 mg.	19	28
		80 mg.	18	25
		80 mg.	15	27
8/5/57	24°C.	0 mg.	25	31
		0 mg.	19	32
		5 mg.	12	34
		5 mg.	13	24
		10 mg.	21	29
		10 mg.	21	28
		20 mg.	15	27
		20 mg.	18	24
		40 mg.	17	28
		40 mg.	15	26
		80 mg.	23	31
		80 mg.	22	29

* All time is to the nearest minute.

TABLE VI (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/10/57	26°C.	0 mg.	19	24
		0 mg.	17	25
		5 mg.	17	24
		5 mg.	17	23
		10 mg.	19	23
		10 mg.	18	21
		20 mg.	18	24
		20 mg.	17	26
		40 mg.	19	23
		40 mg.	21	25
		80 mg.	19	25
		80 mg.	18	21
8/10/57	26°C.	0 mg.	20	22
		0 mg.	20	23
		5 mg.	19	23
		5 mg.	18	22
		10 mg.	20	23
		10 mg.	14	17
		20 mg.	17	23
		20 mg.	20	22
		40 mg.	22	25
		40 mg.	17	22
		80 mg.	14	23
		80 mg.	18	23
8/13/57	28.5°C.	0 mg.	17	27
		0 mg.	19	24
		5 mg.	17	27
		5 mg.	14	24
		10 mg.	20	24
		10 mg.	16	23
		20 mg.	15	25
		20 mg.	16	23
		40 mg.	19	25
		40 mg.	18	26
		80 mg.	13	20
		80 mg.	15	22

* All time is to the nearest minute.

TABLE VII

DAPHNIA DEATH RATE IN VARIOUS CONCENTRATIONS OF LOAM IN DISTILLED WATER, USING ROTENONE AS POISON

Date	Temperature	Concentration per 50 ml.	Time until 5 Daphnia were not swimming*	Time until 5 Daphnia were dead*
7/27/57	27.5°C.	0 mg.	15	18
		0 mg.	19	20
		5 mg.	20	21
		10 mg.	21	24
		20 mg.	21	26
		40 mg.	20	28
		80 mg.	20	23
8/2/57	29°C.	0 mg.	14	18
		0 mg.	14	24
		5 mg.	23	27
		5 mg.	22	24
		10 mg.	21	25
		10 mg.	22	28
		20 mg.	21	26
		20 mg.	24	32
		40 mg.	17	20
		40 mg.	18	20
		80 mg.	18	26
		80 mg.	18	25
8/2/57	27°C.	0 mg.	23	30
		0 mg.	22	28
		5 mg.	24	26
		5 mg.	22	30
		10 mg.	18	28
		10 mg.	23	31
		20 mg.	12	15
		20 mg.	19	29
		40 mg.	22	29
		40 mg.	20	27
		80 mg.	23	26
		80 mg.	28	35

* All time is to the nearest minute.

TABLE VII (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/3/57	27°C.	0 mg.	23	36
		0 mg.	17	19
		5 mg.	13	21
		5 mg.	16	19
		10 mg.	15	21
		10 mg.	14	22
		20 mg.	15	23
		20 mg.	21	30
		40 mg.	13	20
		40 mg.	13	19
		80 mg.	17	25
		80 mg.	18	28
8/9/57	28°C.	0 mg.	12	20
		0 mg.	11	19
		5 mg.	12	22
		5 mg.	12	21
		10 mg.	17	30
		10 mg.	18	29
		20 mg.	18	25
		20 mg.	20	30
		40 mg.	19	30
		40 mg.	15	21
		80 mg.	21	25
		80 mg.	20	33
8/12/57	29.5°C.	0 mg.	7	12
		0 mg.	8	13
		5 mg.	12	18
		5 mg.	12	16
		10 mg.	7	14
		10 mg.	7	14
		20 mg.	9	15
		20 mg.	8	11
		40 mg.	10	17
		40 mg.	8	16
		80 mg.	12	17
		80 mg.	12	16

* All time is to the nearest minute.

TABLE VII (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/14/57	26°C.	0 mg.	15	25
		0 mg.	16	25
		5 mg.	17	20
		5 mg.	18	31
		10 mg.	22	27
		10 mg.	22	28
		20 mg.	20	30
		20 mg.	13	20
		40 mg.	19	22
		40 mg.	23	36
		80 mg.	15	24
		80 mg.	20	29
8/9/57	25°C.	0 mg.	26	41
		0 mg.	27	44
		5 mg.	32	42
		5 mg.	25	29
		10 mg.	23	33
		10 mg.	21	26
		20 mg.	20	31
		20 mg.	25	36
		40 mg.	22	32
		40 mg.	33	55
		80 mg.	33	38
		80 mg.	18	28

* All time is to the nearest minute.

TABLE VIII

DAPHNIA DEATH RATE IN VARIOUS CONCENTRATIONS OF LOAM IN DISTILLED WATER, USING COPPER SULFATE AS POISON

Date	Temperature	Concentration per 50 ml.	Time until 5 Daphnia were not swimming*	Time until 5 Daphnia were dead*
7/12/57	--	0 mg.	11	15
		0 mg.	11	15
		5 mg.	10	14
		5 mg.	12	16
		10 mg.	13	16
		10 mg.	10	15
		20 mg.	9	14
		20 mg.	10	16
		40 mg.	13	17
		40 mg.	12	17
		80 mg.	10	17
		80 mg.	11	17
8/6/57	21.5°C.	0 mg.	14	21
		0 mg.	15	25
		5 mg.	15	19
		5 mg.	14	22
		10 mg.	16	23
		10 mg.	16	21
		20 mg.	16	25
		20 mg.	15	26
		40 mg.	12	25
		40 mg.	17	24
		80 mg.	16	23
		80 mg.	12	28
8/6/57	23°C.	0 mg.	19	30
		0 mg.	16	24
		5 mg.	18	25
		5 mg.	17	22
		10 mg.	14	25
		10 mg.	17	27
		20 mg.	16	24
		20 mg.	16	25
		40 mg.	14	18
		40 mg.	13	21
		80 mg.	14	22
		80 mg.	13	23

* All time is to the nearest minute.

TABLE VIII (Continued)

Date	Temperature	Concentration per 50 ml.	Time until 5 *Daphnia* were not swimming*	Time until 5 *Daphnia* were dead*
8/11/57	27°C.	0 mg.	18	25
		0 mg.	17	27
		5 mg.	17	27
		5 mg.	18	26
		10 mg.	17	24
		10 mg.	18	23
		20 mg.	13	22
		20 mg.	18	24
		40 mg.	20	25
		40 mg.	17	23
		80 mg.	17	24
		80 mg.	10	22
8/11/57	25°C.	0 mg.	21	27
		0 mg.	20	25
		5 mg.	20	28
		5 mg.	21	23
		10 mg.	19	23
		10 mg.	20	24
		20 mg.	19	27
		20 mg.	20	30
		40 mg.	21	26
		40 mg.	20	27
		80 mg.	21	28
		80 mg.	20	25
8/13/57	28°C.	0 mg.	17	28
		0 mg.	14	24
		5 mg.	23	30
		5 mg.	27	31
		10 mg.	19	25
		10 mg.	18	23
		20 mg.	10	24
		20 mg.	9	23
		40 mg.	16	27
		40 mg.	19	27
		80 mg.	20	25
		80 mg.	19	24

* All time is to the nearest minute.

TABLE IX

AVERAGE, STANDARD DEVIATION AND PROBABLE ERROR OF WATER SAMPLES OF VARIOUS CONCENTRATIONS OF SUSPENDED PARTICLES AND ROTENONE

Summary of rotenone and suspended particles of loam

Concentration per 50 ml.	Average death time*	Standard deviation	Probable error
0 mg. of loam	24.5	9.03	6.11
5 mg. of loam	24.47	6.41	4.32
10 mg. of loam	25.33	5.41	3.65
20 mg. of loam	25.27	6.94	4.67
40 mg. of loam	26.13	9.61	6.48
80 mg. of loam	26.53	5.68	3.66

Summary of rotenone and suspended particles of clay

Concentration per 50 ml.	Average death time*	Standard deviation	Probable error
0 mg. of clay	30.5	12.05	8.13
5 mg. of clay	28.57	8.67	5.85
10 mg. of clay	32.07	10.44	7.04
20 mg. of clay	29.07	11.70	7.89
40 mg. of clay	34.71	11.63	7.84
80 mg. of clay	32.07	9.06	6.11

* All time is in minutes.

TABLE X

AVERAGE, STANDARD DEVIATION AND PROBABLE ERROR OF WATER SAMPLES OF VARIOUS CONCENTRATIONS OF SUSPENDED PARTICLES AND COPPER SULFATE

Summary of copper sulfate and suspended particles of loam

Concentration per 50 ml.	Average death time*	Standard deviation	Probable error
0 mg. of loam	23.83	4.50	3.04
5 mg. of loam	23.58	4.99	3.37
10 mg. of loam	22.42	3.40	2.30
20 mg. of loam	23.33	4.23	2.86
40 mg. of loam	23.08	3.73	2.52
80 mg. of loam	23.17	3.34	2.92

Summary of copper sulfate and suspended particles of clay

Concentration per 50 ml.	Average death time*	Standard deviation	Probable error
0 mg. of clay	25.00	4.49	3.03
5 mg. of clay	23.08	4.24	2.86
10 mg. of clay	22.75	4.78	3.12
20 mg. of clay	23.75	2.83	1.91
40 mg. of clay	23.83	3.88	2.62
80 mg. of clay	23.17	4.43	2.99

* All time is in minutes.

the case of rotenone and loam there appears to have been an increase in death time from no suspended material to 80 mg. per 50 ml. of suspended material. However, even in this case the increase was not steady.

Using the standard deviation and probable error as indicators, it can be noted that in copper sulfate poisoning the death time of the organisms was grouped closer about the arithmetic mean death time than in the clay and rotenone combination where the organisms showed a wider range of death time from the average. In the loam and rotenone the deviation from the average was greater than that found in using copper sulfate, but less than that found in clay and rotenone experiments.

The average temperature for rotenone and clay experiments was 25.9°C., and the average temperature of rotenone and loam experiments was 27.3°C. Using these averages and comparing the death time in these two experiments it is evident that in the rotenone and loam suspension at the higher temperatures the average death time was less than in the rotenone and clay suspension of the lower temperatures. In both of the experiments using copper sulfate and either loam or clay the average temperature was 24.9°C. Also in both of these experiments the average death time was similar.

The data indicate that even with this very low concentration of poison, and with the very high amount of suspended materials, not enough poison was being adsorbed to make an appreciable difference in the death time of the experimental organism.

CHAPTER VI

DISCUSSION

The basic purpose of the foregoing experiments was to determine if there was any adsorption of either copper sulfate or rotenone on suspended particles of clay or loam in the water. This adsorption was to be measured indirectly by using the death time of Daphnia. This information might be used to more completely standardize the use of rotenone and copper sulfate in removal of rough fish from ponds and lakes. As these chemicals are now used, often the rough fish are not killed and complete rough fish removal is not assured.

In Chapter III (standardization) it was pointed out that a test tube with distilled water gave a higher reading than an empty test tube. The following seems to account for this change in reading even though more material was between the light source and the light meter. First, the slit was only 3/8ths of an inch wide: thus the test tube overlapped the slit on both sides. The index of refraction of water and glass is such that the light hitting the overlapped part of the test tube was directed into the slit and thus caused a higher light meter reading. When water was not in the test tube, the light hitting the overlapped part of the test tube was not directed into the slit, but rather it hit the side

of the box and had no influence upon the light meter.

The turbidity of the sample of water taken from the lake was noted to vary from place to place and from time to time. This difference was due to a number of factors such as size of suspended materials, organic substances, dissolved materials and wind and current.

By using a very high concentration of suspended matter, and a comparatively low concentration of poison, any adsorption that was going to take place should have shown up quite quickly. In only one case was there any fairly consistent indication that adsorption took place: this was with the use of loam and rotenone. Even here the total difference between a concentrated amount of suspended material and no suspended material was less than two (2) minutes. Also, it should be noted that a steady increase in death rate, as the concentration of loam increased, did not appear.

A control was maintained for each of the experiments, and in each case the control organism outlived the test organism.

The data in Chapter V raise the possibility that as the temperature increased, time needed to kill the Daphnia decreased. A relationship between increase in temperature and decrease in death time for Daphnia would be in accordance

with the findings of others.[1] Some experimenters have found that the metabolic rate, velocity of aging, heart rate and susceptibility to different poisons vary in the same direction as the temperature.[2]

Tables IX and X show that there was a wide variation in death time of the organism, especially where clay and rotenone were used in combination. In this problem no effort was made to use one size of organism, or one sex: however, not all large or older Daphnia were used in any one experiment. This wide variation noted in death time appears to be consistent with the findings of Mac Arthur and others.[3]

Before putting too much import in the apparent adsorption of rotenone on suspended particles of loam these things should be considered: First, the difference between average death time of Daphnia in no suspended loam to a saturated solution was only two (2) minutes. Second, the increase in death time was not a steady increase as one would expect. Third, difference in age, sex and temperature could

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[1]Hoffman, Donald, and Rigkalla Zakhary, "The Effect of Temperature on the Molluscacidal Activity of Copper Sulphate," Science, 114:521-3 Nov. 16, 1951.

[2]Mac Arthur, op. cit., pp. 243-258.

[3]Ibid.

possibly account for the slower death time at the higher concentration of loam. Taking these above facts into consideration it seems likely that the slower death rate in the higher concentration of suspended materials was due to something other than the adsorption of the poison upon the suspended matter.

In conclusion it can be said that the temperature and concentration of rotenone and copper sulfate are far more important in the use of these as poisons than the amount of clay or loam particles suspended in the water. In only one experiment was there any fairly consistent indication of adsorption. Effects of other possible factors and the amount of apparent adsorption make the importance of the apparent adsorption negligible.

CHAPTER VII

SUMMARY

The kill in fish poisoning is not uniform with similar concentrations of poisons. From this developed the problem of possible adsorption of poison on suspended matter in the water.

A history of investigations performed in the field of rough fish removal indicated no work had been carried out concerning the possible adsorption of rotenone or copper sulfate.

The method of determining turbidity and standardization of procedures is set forth in Chapter IV. Death time is described as no heart beat and absence of movement of the thoracic appendages of the Daphnia.

The collection of data was based on the use of 3,190 Daphnia. The data are placed in table form, and interpretation of the information is given, followed by a discussion as to the meaning and possible importance of the data.

As determined in the study the influence of adsorption of poison on either clay or loam was negligible.

Since the adsorption of poison on suspended particles of clay or loam were negligible, these factors need not be considered in fish control, where the concentration of poison used is far above the concentration used in this problem.

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